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Determination of the Chromosomal Position of Three Mutations Using Recombination Mapping in Drosophila Melanogaster

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DETERMINATION OF THE CHROMOSOMAL POSITION OF THREE MUTATIONS USING RECOMBINATION MAPPING IN *DROSOPHILA MELANOGASTER*

by
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ABSTRACT

Genetic screens are performed in order to characterize genes and mutations, often relating to developmental processes. A previous genetic screen performed in *Drosophila melanogaster* found three mutations on the second chromosome affecting glial cell development: FF12, DD68, and D19. These mutations each disrupted the pattern and expression of *reversed polarity* (*repo*), a target gene of the glial fate master regulator, *glial cells missing* (*gcm*). Here, I use recombination mapping with phenotypically observable dominant markers to locate the genetic map position of each mutation. This method provides a rapid estimate of the mutations' locations that can then be tested against molecularly-defined deficiencies to pin-point a precise position. FF12, DD68, and D19 were all determined to be on chromosome arm 2R.

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LIST OF ABBREVIATIONS

EMS	ethyl methane sulphonate
mRNA	messenger RNA
gcm	*glial cells missing* gene
Gcm	glial cells missing protein
repo	*reversed polarity* gene
Repo	reversed polarity protein
CNS	central nervous system
BDSC	Bloomington *Drosophila* Stock Center
CyO	curly
S	*star*
wg	*wingless*
Sp	*sternopleural*
Bl	*bristle*
L	*lobe*
P	parental cross

CHAPTER 1

BACKGROUND AND INTRODUCTION

1.1: Genetic Screens in *Drosophila*

Genetic screens are used to select for individuals that possess a specific phenotype in a mutagenized population (St. Johnston, 2002). The phenotype observed from a mutation can provide insight into the function of a gene. Performing genetic screens in *Drosophila* has proven to be useful because of the many developmental processes that have been conserved between flies and vertebrates (St. Johnston, 2002). Famously, Hedgehog and the vertebrate homolog Sonic Hedgehog have similar functions in limb patterning. Additionally, 197 of 287 human disease genes contain a homolog in *Drosophila* (St. Johnston, 2002). The ability to easily perform genetic screens in *Drosophila* can, therefore, be analyzed not only to understand each gene's role within the fly but also its vertebrate counterpart (St. Johnston, 2002).

In order to carry out genetic screens, scientists had to create a way to generate mutations. In 1968, Lewis and Bacher described the use of ethyl methane sulphonate (EMS) to induce mutations in *Drosophila* (Lewis and Bacher, 1968). Still, the most commonly used mutagen in *Drosophila*, EMS, an alkylating agent, is fed to flies to induce a high occurrence of point mutations in their DNA. Point mutations can result in missense or nonsense mutations causing a disruption in gene function (St. Johnston, 2002). Therefore, the prevalence of mutations in a gene is dependent on the size of the

gene's coding regions and the number of critical aminio acids that it contains (Greenspan, 2004).

Traditional genetic screens have been used to identify mutations that affect embryo patterning. Winning a Nobel prize for their work, Christiane Nüsslein-Volhard and Eric Wieschaus performed a mutagenesis screen finding most of the mutations in genes which are essential to patterning in *Drosophila*'s development (Nüsslein-Volhard and Wieschaus, 1980). The model organism has key features that allowed for success when Nüsslein-Volhard and Wieschaus searched for these developmental mutations. One, *Drosophila* has an exoskeleton which allows the patterning of the embryo to be visualized. Two, few mutations prevent embryonic development at early stages because the mother passes maternal mRNA to the egg eliminating the need for the embryo's genes to be transcribed for patterning (St Johnston, 2002).

Although major accomplishments have been achieved with traditional screens, they do have disadvantages. One limitation of traditional genetic screens is they are only capable of identifying a mutation's earliest phenotype. This limitation is applicable to several vital proteins passed maternally to the embryo (e.g. Wingless) (St Johnston, 2002). The zygotic phenotype of these genes in homozygous mutants is only visible when the maternal protein supply dissipates. Since this is a gradual process, later functions or phenotypes of the target gene cannot be analyzed. New screens have been developed to bypass this issue such as enhancer and suppressor screens and clonal screens (St Johnston, 2002).

1.2: Mapping Mutations

A key feature of *Drosophila* is their polytene chromosomes. Polytene chromosomes are formed from non-disjunction occurring in individual chromosomes that have sustained multiple rounds of replication (Zhimulev et. al., 2016). They have clear, distinctive banding patterns, making it possible to correlate genetic map positions (expressed in centiMorgans) with physical features of the chromosomes (Greenspan, 2004). This has allowed DNA sequences to be mapped to specific physical locations on the chromosome (expressed by numbered segments). Banding pattern is a ubiquitous organization convention, common to both polytene and normal, non-polytene, chromosomes. The universality provided by these chromosomes warrants them as the prototype for eukaryotic interphase chromosomes and makes them beneficial for mapping (Zhimulev et. al., 2016).

Knockout mutations are essential in understanding the cellular function of genes (Kahsai and Cook, 2018). In addition, different mutations such as those that affect levels of gene expression or protein activity can provide insight that knockout mutations cannot. Many mutations present in *Drosophila* have been characterized phenotypically, but these mutations have not been identified with sequence-defined genes. These stocks are possibly beneficial but rarely sought after by geneticists. Typically, geneticists researching specific sequence-defined genes or processes focus on mutations that are associated with that specific gene, not considering the unmapped mutations as possible alleles. Therefore, mapping mutations can lead to new, more holistic understandings of related genes' functions (Kahsai and Cook, 2018).

Simple techniques creating chromosomal deletions with breakpoints known at the single nucleotide level are being used to map mutations to very precise positions on the chromosome (Kahsai and Cook, 2018). *Drosophila* now has nearly complete genomic deletion coverage (>98%) and subdivisions between breakpoints, more than any other multicellular organism (Cook et. al., 2012). While blindly using deletion complementation as a mapping method can be slow and labor intensive, homologous chromosome recombination can be taken advantage of to advance the mapping process (Sapiro et. al., 2013). Recombination analysis is a reliable mapping method that has been used for over 100 years. In order to obtain a location from recombination methods, the frequency of chromosomal exchange is compared to a reference locus. The resolution of recombination mapping is positively correlated with the density of markers within the stock and the number of recombinant progeny examined. Many methods are used to achieve a high density of markers such as single-nucleotide polymorphisms, molecularly defined P-elements, and recessive markers. While all effective, these methods of recombination mapping are all labor intensive (Sapiro et. al., 2013).

One method of recombination mapping that provides less labor-intensive and more rapid results is the use of pairs of dominant, phenotypically visible markers. This method reduces the number of crosses and generations necessary to estimate genetic map positions (Sapiro et. al., 2013). Since easily visible dominant markers are infrequent, this method does not produce a high-resolution map, rather an approximation of the mutation's location. When paired with subsequent steps of complementation testing with deficiencies in the area of the mutation, the actual physical location can be determined (Sapiro et. al., 2013).

Recombination can be an effective tool in genetics research in flies because there is a complete absence of recombination in males. With recombination only occurring in females, and balancer chromosomes, a necessary component to successfully perform screens without crossing over, the transmission of chromosomes to progeny can be traced unambiguously. Balancer chromosomes are inverted chromosomes that suppress meiotic crossing over and prevent crossover products (Greenspan, 2004). Balancers complete this task by producing recombinant chromatids that do not segregate normally in the first meiotic division. These balancer chromosomes carry dominant marker alleles that allow for visual identification of flies possessing the balancer, and most balancers contain recessive lethal mutations that inhibit the balancer from appearing homozygous in a stock (Greenspan, 2004). Specifically, on *Drosophila*'s second chromosome, commonly used balancers include SM5 and CyO (Miller et. al., 2016).

1.3: Mutations of Interest

Proper nervous system development requires appropriate gene specification and strict organization of many neural cells. One of these neural cell types is glia. Glia have many roles in the developing nervous system: they balance neural stem cell proliferation, control the differentiation of neural precursors, ensheath neurons, consume neural waste produced during development, and advance synapse establishment and maturation (Stork et. al., 2012). In order to achieve these roles, neural precursors must first differentiate into glia. A master regulator of glial cell fate in *Drosophila* is the *glial cells missing* (*gcm*) gene. The protein product of *gcm* is a DNA-binding transcription factor required for the development of almost all glial cells in *Drosophila*. When *gcm* is expressed, neural cells

have a glial fate, and when absent, neural cells take on a neuronal fate (Jones, 2008). Homologs of *gcm* in mammals have been identified with conserved molecular properties. In both *Drosophila* and the vertebrate nervous system, glial cell differentiation is closely related to neurogenesis. With the preservation of similar genes between species, comparable mechanisms of gliogenesis may be seen in *Drosophila* and vertebrates (Jones, 2008).

In order to completely understand gliogenesis, mutations influencing the process must be observed and characterized. Relevant mutations were discovered in a genetic screen in Dr. Brad Jones' lab using EMS (Jones, 2008). The main objective of this screen was to discover genes affecting glial cell patterning, positioning, migration, function, and other elements of differentiation. This was accomplished using antibody staining on the glial-specific protein, Reversed Polarity (Repo). While Gcm is pertinent to differentiating neural precursors to glial cells, its expression fades during the embryonic phase (Stork et. al., 2012). For that reason, the steadily expressed Repo is stained. A transcriptional target of Gcm, *repo* is a gene in *Drosophila* expressed in all glial cells excluding midline glia which makes it an excellent marker for glial cells (Jones, 2008).

Drosophila embryos are well equipped for techniques that allow for concise in vivo studies of glial-expressed genes in central nervous system (CNS) development. Common techniques used for this purpose are immunohistochemistry, in situ hybridization, and live imaging which all take advantage of tissue transparency in the embryo (Stork et. al., 2012). Jones used these techniques to find each mutant—D19, DD68, and FF12—while examining hundreds of lines from the mutagenesis screen

(Jones, 2008). These mutants were stained with anti-Repo using immunohistochemistry to visualize the glia during the embryonic stage (Figure 1).

Mutations of interest—those affecting *repo* expression and ultimately glia—were analyzed for their effect on glial cell development and their relationship with *gcm*. As shown in Figure 1, all three mutations are embryonic lethal as homozygotes and show irregular patterns of glia in their CNS. Accordingly, these mutants are all candidates for further characterization. In this study, I use recombination mapping to locate the position of three target mutants on *Drosophila*'s second chromosome: D19, DD68, and FF12.

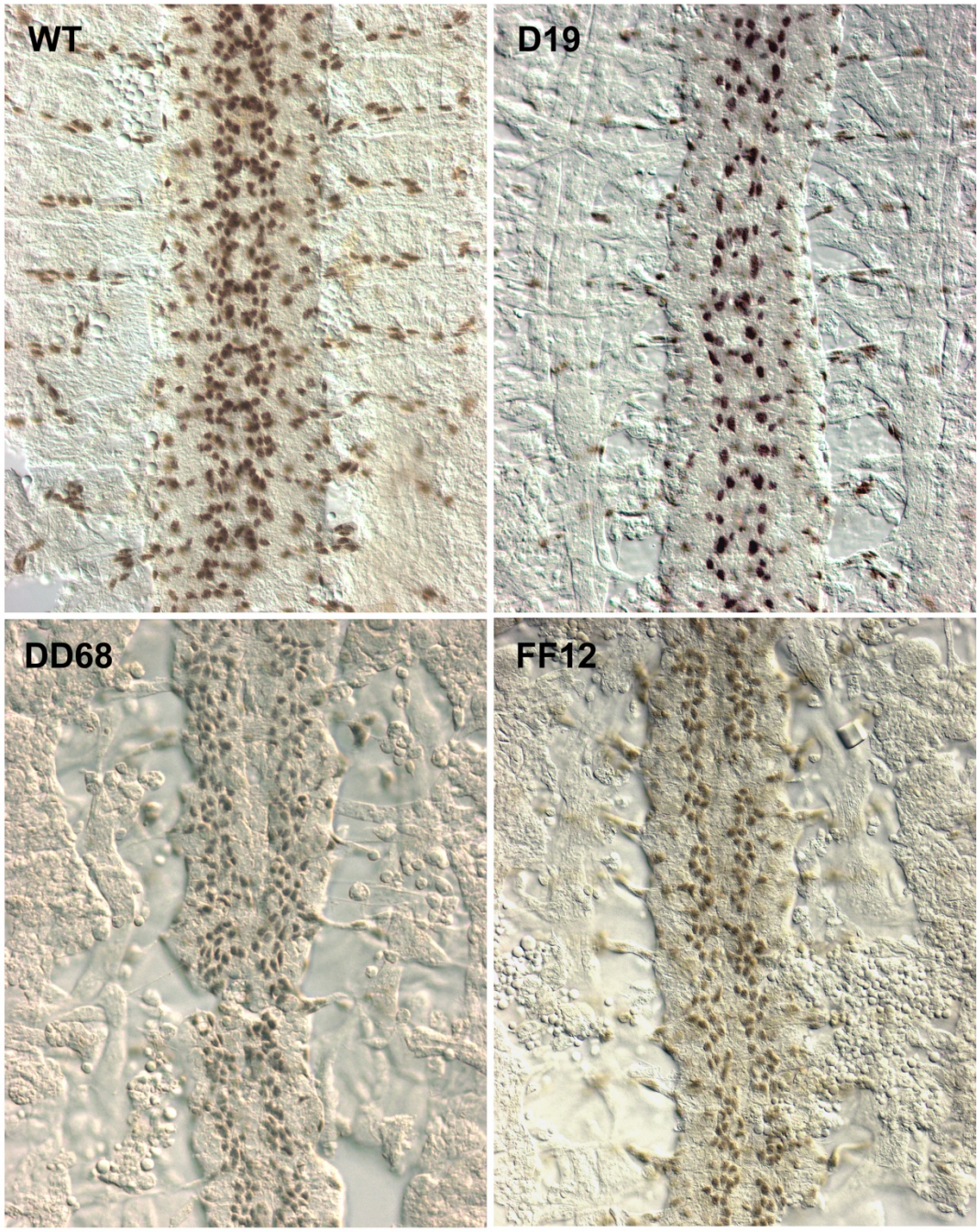

Figure 1. Mutant embryos stained with Repo monoclonal antibody. The anti-Repo stain of mutants D19, DD68, and FF12 makes the glial pattern abnormalities visible when compared to the wild type (WT). Anterior is up. The embryos pictured are homozygous for their mutations.

CHAPTER 2

MATERIALS AND METHODS

2.1: *Drosophila* husbandry

The following mutated stocks were generated in a genetic screen using EMS: D19, DD68, and FF12. Stocks containing dominant markers were collected from BDSC: 389, 1366, and 5194. Stock 389 contained dominant markers *S[1]* and *wg[Sp-1]*; stock 1366 contained dominant markers *wg[Sp-1], Bl[1],* and *L[rm]*; stock 5194 contained dominant markers *L[2]* and *Pin[1]*. Table 1 displays these markers with necessary mapping material. All flies were cultivated on cornmeal molasses media with yeast.

Genotype	**Name**	**cM**	**Cytology**
S[1]	*Star, Asteroid*	2-1.3	21E4
wg[Sp-1]	*Sternopleural*	2-22	27F1
Bl[1]	*Bristle*	2-54.8	38B5
L[rm] or L[2]	*Lobe*	2-72	51A4
Pin[1]	*Pin*	2-107.3	60C6-D1

Table 1. Valuable dominant markers expressing observable phenotypes on *Drosophila*'s second chromosome for mutation mapping (adapted from Sapiro et. al., 2013).

2.2: Scoring Markers

Crosses were performed at 25°C with three to six virgins and two to six males per cross. Nine crosses were set up in order for each mutation to be crossed with dominant markers that spanned the entire chromosome. Therefore, stocks D19, DD68, and FF12 were each crossed with stocks 389, 1366, and 5194. Initially, mutant virgins were crossed with dominant marker males. The F1 progeny produced from these crosses were then examined for the presence of the balancer chromosome, CyO (curly wings). Virgin females lacking the balancer (i.e. females with straight wings) were collected for the second cross with mutant males. The resulting F2 progeny were then scored for the presence of dominant markers and the absence of the balancer (Figure 2). The phenotypes of each dominant marker are: *Star* (*S*), scored for smaller, narrower eye with a rough and rounded texture; *Sternopleural* (*Sp*), scored for extra bristles on the sternopleurite; *Bristle* (*Bl*), scored for short, thick bristles in the thoracic region; *Lobe* (*L*), scored for reduced eye size; and *Pin* (*Pin*), scored for short, thick bristles thoracic bristles. The F2 progeny that are relevant to this mapping method are those that have either lost one or both dominant markers in a pair (Figure 3). For instance, when the pair *S,Sp* is examined, the only progeny necessary to determine the map location are *S*,+, +,*Sp*, and +,+ (Sapiro et. al., 2013).

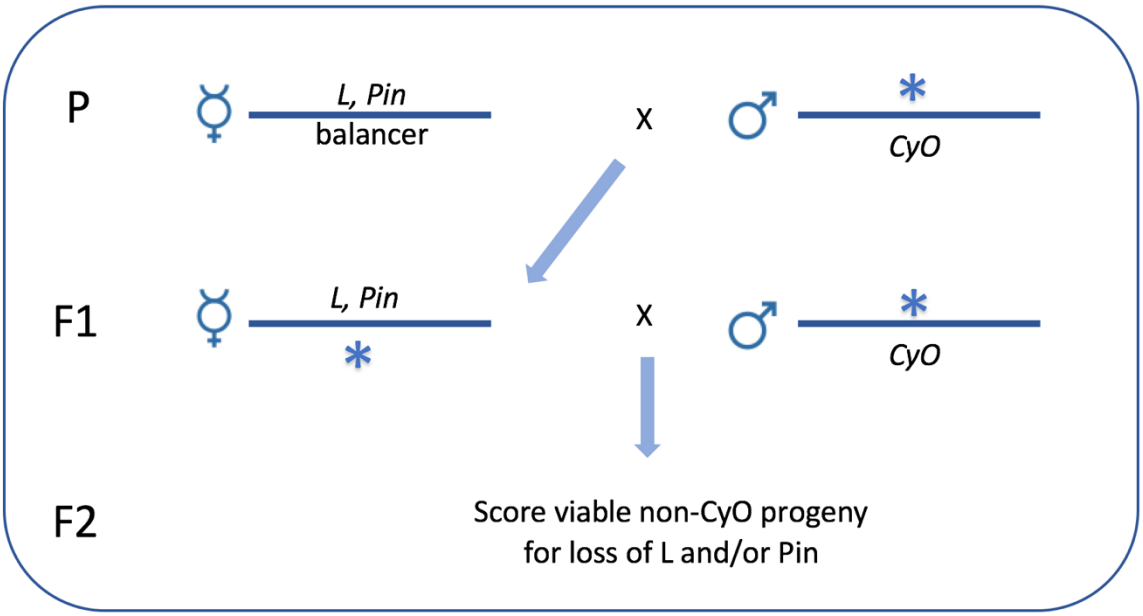

Figure 2. Recombination Mapping Scheme. In the parental cross (P), mutant *Drosophila* were crossed with a stock containing dominant markers on the second chromosome. The resulting progeny were screened for females containing the dominant markers and the chromosome carrying the mutation of interest (no balancer, CyO, progeny). These females were backcrossed with males from the mutant stock for the F1 cross. The resulting progeny are scored for the absence of CyO and loss of *L* and/or *Pin* (Adapted from Sapiro et. al., 2013).

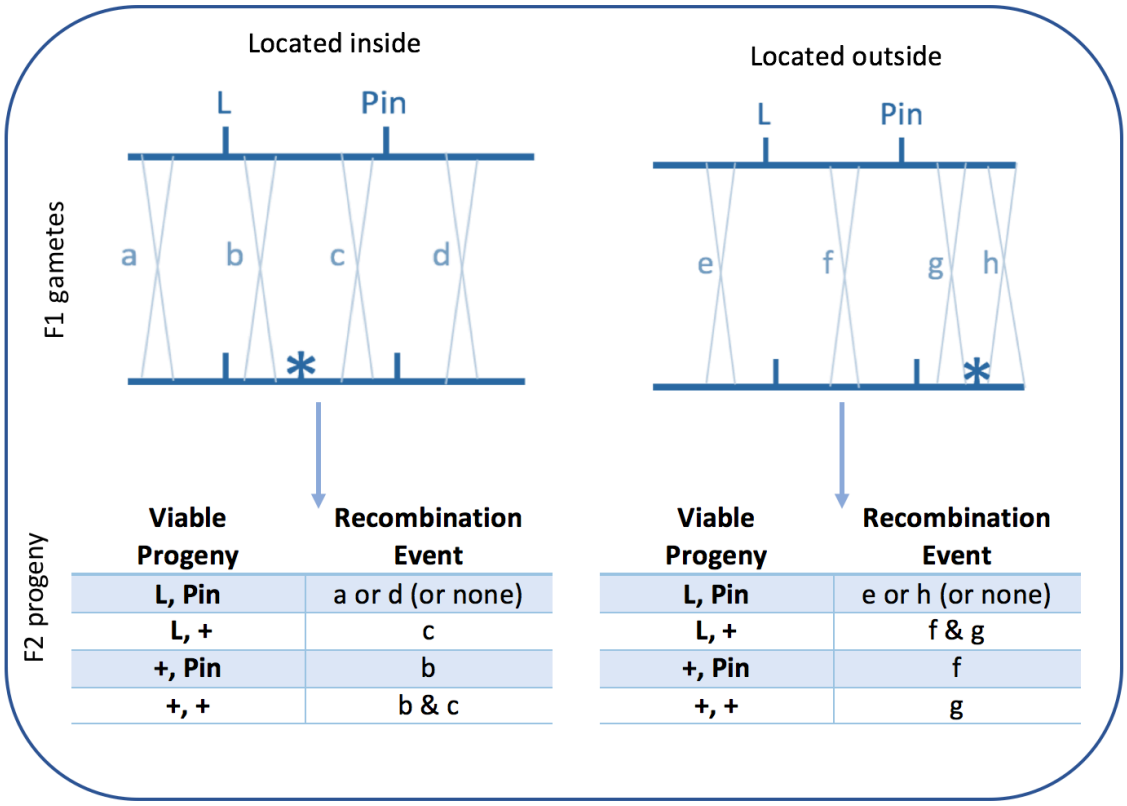

Figure 3. Possible Recombination Events. Potential recombination events between the mutant and dominant marker chromosomes contingent upon the mutation being inside or outside of the pair of markers. If recombination occurs in "a" or "d," the recombinant chromosome is viable with progeny containing both markers (*L, Pin*). If recombination occurs in "b," the recombinant chromosome containing only *L* is lethal and the recombinant progeny with *Pin* are viable (+, Pin progeny). The opposite is true for "c," resulting in *L*, + progeny. The ratio of these recombination events indicates the approximate position of the mutation. As in the first example, a recombination event occurring in "e" or 'h" results in viable *L, Pin* progeny. A recombination event in "f" produces only +, *Pin*. *L*, + progeny are only possible if double recombination occurs at "f" and "g" (adapted from Sapiro et. al., 2013).

The viable recombinant progeny in the F2 generation that have lost one or both markers are counted to obtain a ratio from them. If unmarked progeny are absent or infrequent, then the mutation of interest is located within the two markers and the ratio of the splits (*S*,+ and +,*Sp*) determines the approximate position of the mutation between the markers (Figure 4). If unmarked progeny are frequent or common, then the mutation is located outside of the markers. The ratio of the splits, in this case, would be used to determine the direction of the mutation compared to the markers. So if *S*,+ is more frequent than +,*Sp*, then the mutation is on the outside side of *S* (to the left) rather than *Sp* (to the right), and vice-versa.

$$\frac{\begin{pmatrix} loss\ of\ left \\ marker \end{pmatrix}}{\begin{pmatrix} loss\ of\ left \\ marker \end{pmatrix} + \begin{pmatrix} loss\ of\ right \\ marker \end{pmatrix}} \times \begin{pmatrix} cM\ between \\ markers \end{pmatrix} + \begin{pmatrix} cM\ of\ left \\ marker \end{pmatrix} = \begin{pmatrix} map \\ position \\ cM \end{pmatrix}$$

Figure 4. The Formula for Genetic Map Position. Once the mutation is determined to be inside the markers, the number of F2 progeny that have lost one marker are counted and used in this equation to find the approximate location (adapted from Sapiro et. al., 2013).

The genetic map position obtained from recombination analysis then corresponded with the physical position or chromosomal cytology. Since there is not a linear relationship between genetic and physical positions, each mutation's estimated location was compared with known information about the position of local genes (Sapiro et. al., 2013). This information was found using the cytogenetic map published in *The*

Genome of Drosophila melanogaster as well as information made available on FlyBase Gene Reports. (Lindsley and Zimm 1992, Marygold et al. 2013).

CHAPTER 3

RESULTS

Estimated chromosomal locations were determined for all three mutations: FF12, DD68, and D19. My recombination mapping method tracked the lethal phenotype of these mutations. After estimated genetic map locations were found, they were converted into physical map locations using a cytogenic map and conversion table available on FlyBase.

Mutation	**cM**	**cyto**
FF12	76.7	52E
DD68	87.7	56B
D19	72	51B

Table 2. Estimated chromosomal location of mutations.

As shown in Figure 5, each mutation's location for all three lines was found when crossed with stock 5194 within the interval of dominant markers *L* and *Pin*. This was determined by the lack of flies without either dominant marker. For FF12, DD68, and D19 there were no non-balancer F2 progeny missing both *L* and *Pin*. The crosses between stocks 389 and 1366 and the mutant stocks mainly indicated that the mutation was in the direction of *L, Pin* region by having more flies present with the dominant marker on the right than on the left, with the exception *wg[Sp-1], Bl[1]*. In both DD68 and FF12, there were more *wg[Sp-1]* than *Bl[1]*. This is likely due to *Bl* (54.8 cM) being located near the centromere (~55 cM) (Lindsley and Zimm, 1992). The centromere

causes inhibitory effects on recombination rates, and therefore makes recombination an inefficient way to map adjacent mutations (Sapiro et. al., 2013).

The D19 x 389 cross did not produce any viable nonbalancer F1 progeny, so the second cross was unable to be conducted and analyzed. While this cross with dominant markers *S* and *wg[Sp-1]* was unable to be falsified, there is empirical evidence in support of mutation D19 being located between *L, Pin*. As shown in Figure 5, the *Bl*, *L[rm]* split suggests that the mutation is to the right of *L[rm]*. Also, the *L, Pin* split did not have any nonbalancer F2 progeny that lacked both *L* and *Pin*—the key indicator of the mutation being within the markers.

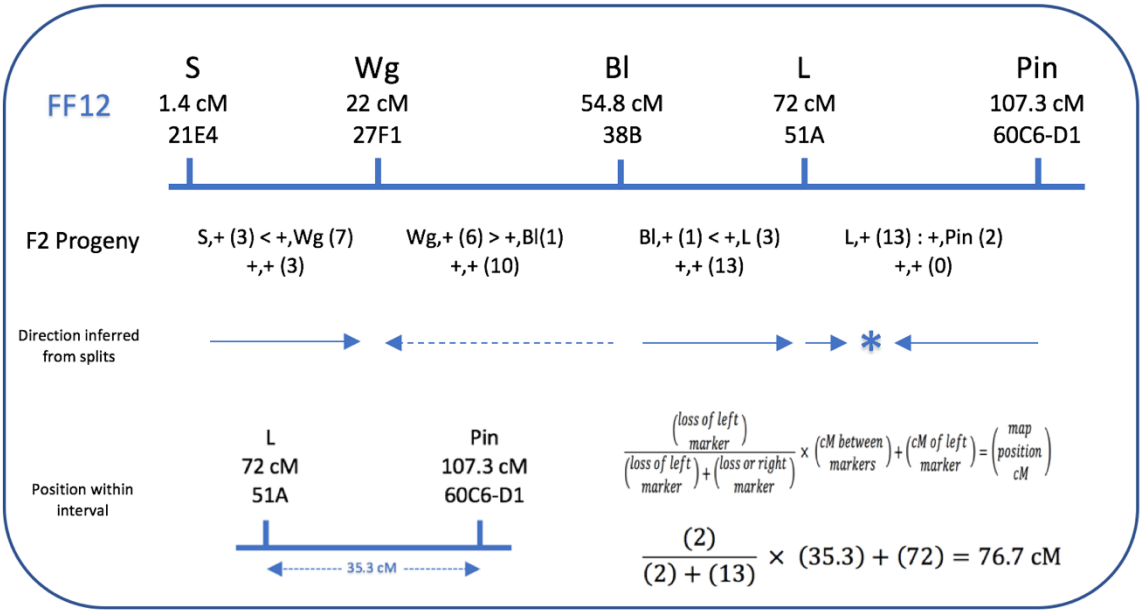

Figure 5A. The Process of locating mutation FF12. (Adapted from Sapiro et. al., 2013).

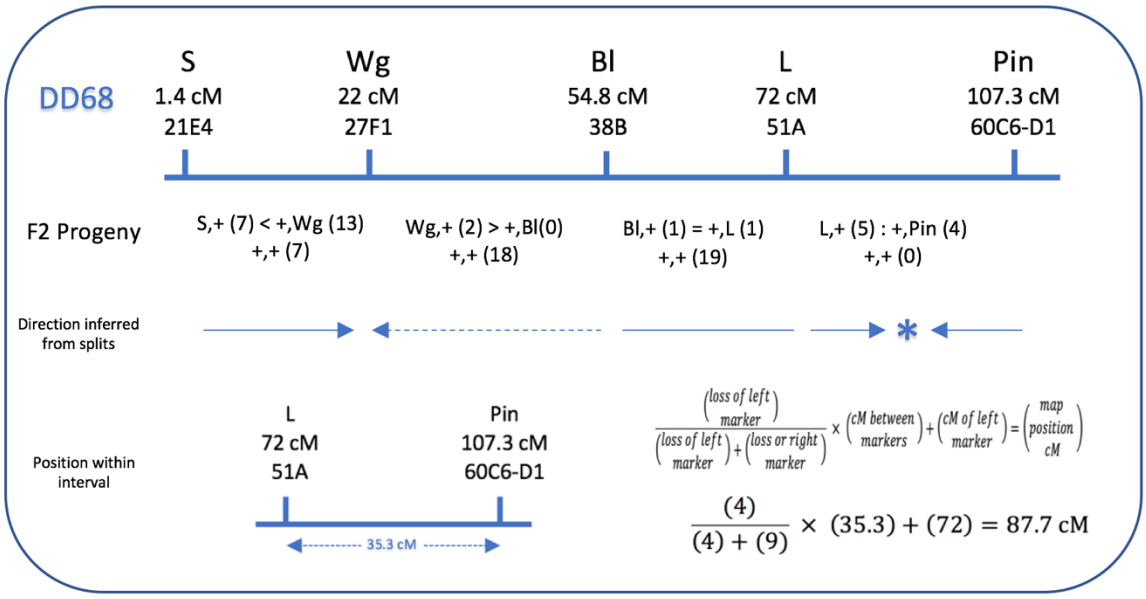

Figure 5B. The Process of locating mutation DD68. (adapted from Sapiro et. al., 2013).

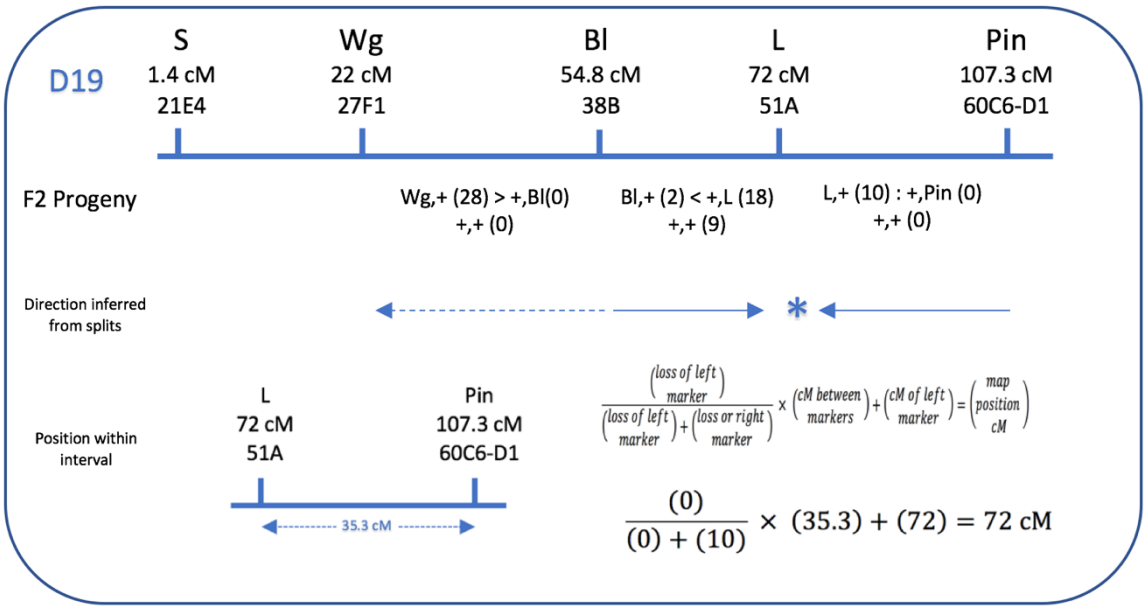

Figure 5C. The Process of locating mutation D19. (adapted from Sapiro et. al., 2013).

CHAPTER 4

DISCUSSION

The results obtained from recombination mapping are only approximations (Sapiro et. al., 2013). In order to determine an exact location from this point, deletion mapping must be used. In deletion mapping, fly stocks that have molecularly defined deficiencies are crossed with the mutants (Kahsai and Cook, 2018). Wherever the mutant fails to complement is the location causing the lethal phenotype. It is important to verify embryonic phenotypes in mutant over deficiency embryos by staining for Repo. Since the approximate location has now been discovered, only deficiencies spanning the region around the estimated location are needed. The following deficiencies ranging 51B-53D are needed to adequately test the region around FF12 (~52E): Df(2R)ED2419, Df(2R)ED2436, Df(2R)ED2486, and Df(2R)ED2522. The following deficiencies ranging 55A-57A are needed to adequately test the region around DD68(~56B): Df(2R)ED3610, Df(2R)ED3683, Df(2R)Exel6069, Df(2R)BSC135, Df(2R)ED3716, Df(2R)BSC782, Df(2R)ED3728, and Df(2R)ED3737. Finally, the following deficiencies ranging 49E-52E are needed to adequately test the region around D19(~51B): Df(2R)CX1, Df(2R)BSC361, Df(2R)ED2354, Df(2R)ED2419, and Df(2R)ED2436. These deficiencies can be obtained from BDSC. If the deficiencies complement the entire region of D19's location, the D19 x 389 cross should be performed again to falsify this area as the possible mutation position. The accuracy of recombination mapping can be improved by increasing the number of flies used in each cross. This would allow for a

greater F2 generation to be produced and scored, directly increasing the accuracy of position estimates. Mapping resolution could also be improved by increasing the density of dominant markers with which the mutants are crossed.

Once an exact position of FF12, DD68, and D19 is found with complementation testing, the mutations should then be associated with sequence-defined genes. We assume theses mutations are homozygous lethal. If the results turn out to be inconclusive, then this assumption should be tested. Some possible genes affecting nervous system development that FF12 could be linked to based on its estimated location are *spinster*, *dystroglycan*, and *aspartyl β-hydroxylase*. DD68's estimated location also overlapped with a gene that has a role within the nervous system: *enabled,* as found on FlyBase. While these are just a few of many possible genes the mutations could affect, it confirms the presence of neural-related genes within the regions around the mutations. Mutations are typically studied when they are related to a gene, therefore completing the mapping process will allow the mutations to be sought after more often and further researched. This could possibly show that the mutation is an allele of a gene. Ultimately, the mutations' positions could provide a novel and more comprehensive perspective of a gene and its function.

Mutants FF12, DD68, and D19 all exhibited pattern and expression errors when stained for Repo. Thus, these mutants are suspected to affect genes necessary for gliogenesis. The mutants could disturb *gcm* or a known target gene such as *repo*; on the other hand, they could disturb a novel gene and lead to the discovery of new *gcm* target genes. In order to determine the extent of the mutations' effects, the next step would be to stain the mutant lines with a series of antibodies (i.e. anti-Engrailed, anti-Even-skipped,

etc.) against indicators in the CNS and peripheral nervous system to characterize a greater depth of pattern formation, neural specification, and axon pathfinding. These antibody staining procedures should reveal whether each mutant's defect in gliogenesis is confined to glial cells, or a more broad developmental process (Jones, 2008).

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